

**WHAT IS CLAIMED IS:**

1. A method for synthesizing an RNA oligonucleotide comprising combining a single-stranded circular oligonucleotide template comprising at least one copy of a nucleotide sequence complementary to the sequence of the desired RNA oligonucleotide with an effective amount of at least two types of ribonucleotide triphosphate and an effective amount of a polymerase enzyme to yield a single-stranded RNA oligonucleotide multimer complementary to the circular oligonucleotide template, wherein the RNA oligonucleotide multimer comprises multiple copies of the desired RNA oligonucleotide.
2. The method of claim 1 wherein the nucleotide sequence of the circular oligonucleotide template is devoid of an RNA polymerase promoter sequence.
3. The method of claim 1 wherein the circular oligonucleotide template has about 15-1500 nucleotides.
4. The method of claim 1 performed in the absence of an oligonucleotide primer.
5. The method of claim 1 performed without the addition of auxiliary proteins.
6. The method of claim 1 wherein the polymerase enzyme is selected from the group consisting of T7 RNA Polymerase, T4 RNA Polymerase, SP6 RNA Polymerase, RNA Polymerase II, RNA Polymerase III, T3 RNA Polymerase, *E. coli* RNA Polymerase and homologs thereof having at least about 80% homology.

7. The method of claim 6 wherein the polymerase enzyme is selected from the group consisting of T7 RNA Polymerase, T4 RNA Polymerase, SP6 RNA Polymerase, RNA Polymerase II, RNA Polymerase III, T3 RNA Polymerase and *E. coli* RNA Polymerase.

8. The method of claim 1 wherein the RNA oligonucleotide multimer comprises multiple copies of a cleavage site.

9. The method of claim 1 wherein the circular oligonucleotide template comprises DNA.

10. The method of claim 1 wherein the RNA oligonucleotide multimer has at least 1000 nucleotides.

11. The method of claim 10 wherein the RNA oligonucleotide multimer has at least 5000 nucleotides.

12. The method of claim 1 wherein the RNA oligonucleotide multimer is biologically active.

13. The method of claim 12 wherein the RNA oligonucleotide multimer is catalytically active.

14. The method of claim 12 wherein the RNA oligonucleotide multimer comprises multiple copies of a ribozyme.

15. The method of claim 14 wherein the ribozyme is capable of *trans* cleavage.

16. The method of claim 1 further comprising cleaving the RNA oligonucleotide multimer to yield multiple copies of the desired RNA oligonucleotide.

17. The method of claim 16 wherein the cleavage is autolytic.

18. The method of claim 16 wherein the desired RNA oligonucleotide comprises a ribozyme.

19. The method of claim 16 wherein the desired RNA oligonucleotide is linear.

20. The method of claim 16 wherein the desired RNA oligonucleotide is circular.

21. The method of claim 16 wherein the desired RNA oligonucleotide is capable of intramolecular ligation.

22. The method of claim 21 wherein the desired RNA oligonucleotide comprises a hairpin-type ribozyme.

23. The method of claim 16 wherein the desired RNA oligonucleotide is biologically active.

24. The method of claim 23 wherein the biologically active RNA oligonucleotide comprises a catalytic RNA, an antisense RNA, or a decoy RNA.

25. The method of claim 23 wherein the biologically active RNA oligonucleotide comprises a catalytic RNA.

26. The method of claim 23 wherein the biologically active RNA oligonucleotide has endonuclease, exonuclease, polymerase, ligase, phosphorylase, dephosphorylase, or protease activity.

27. The method of claim 23 wherein the biologically active RNA oligonucleotide comprises a ribozyme.

28. The method of claim 27 wherein the ribozyme is a hairpin, a hammerhead-motif, or a hepatitis delta catalytic ribozyme.

29. The method of claim 23 wherein the biologically active RNA oligonucleotide cleaves a disease-associated RNA, DNA, or protein.

30. The method of claim 16 wherein cleavage of the RNA oligonucleotide multimer is effected chemically or by contact with a site-specific endonuclease.

31. The method of claim 30 wherein cleavage is effected by a site-specific endonuclease comprising a protein or a ribozyme.

32. The method of claim 16 wherein the desired RNA oligonucleotide has well-defined ends.

33. The method of claim 1 or 16 performed inside a cell.

34. The method of claim 33 wherein the cell is a plant cell or an animal cell.

35. The method of claim 33 wherein the cell is a human cell.

36. The method of claim 33 wherein the circular oligonucleotide is introduced into the cell using direct injection, electroporation, calcium phosphate treatment, lipid-mediated delivery, or cation-mediated delivery.

37. The method of claim 33 wherein the circular oligonucleotide has 15-1500 nucleotides.

38. The method of claim 33 wherein the cell is transfected with a gene encoding an effective RNA polymerase operably linked to a promoter.

39. The method of claim 38 wherein the RNA polymerase is T7 or *E. coli* polymerase.

40. The method of claim 33 performed *ex vivo*.

41. The method of claim 33 performed in a cell explanted from a plant or animal.

42. The method of claim 34 wherein the cell is reimplanted into the plant or animal after synthesis of the RNA oligonucleotide multimer.

43. The method of claim 41 wherein the animal is a human.

44. The method of claim 33 performed *in vivo*.

45. The method of claim 33 performed in cell culture.

46. An RNA oligonucleotide multimer synthesized according to the method of claim 1.

47. The RNA oligonucleotide multimer of claim 46 which is biologically active.

48. The biologically active RNA oligonucleotide multimer of claim 47 comprising a ribozyme.

49. An RNA oligonucleotide synthesized according to the method of claim 16.

50. The RNA oligonucleotide of claim 49 which is biologically active.

51. The biologically active RNA oligonucleotide of claim 50 comprising a ribozyme.

52. A method for synthesizing an RNA oligonucleotide comprising contacting the cell of a living organism *in situ* with a single-stranded circular oligonucleotide template comprising at least one copy of a nucleotide sequence complementary to the sequence of the desired RNA oligonucleotide, such that the circular oligonucleotide is taken up by the cell and processed intracellularly to yield an RNA oligonucleotide multimer comprising multiple copies of the desired RNA oligonucleotide.

53. The method of claim 52 wherein the circular oligonucleotide has about 15-1500 nucleotides.

54. The method of claim 52 wherein the living organism is a plant or an animal.

55. The method of claim 52 wherein the living organism is a human.

56. The method of claim 52 wherein the RNA oligonucleotide multimer is cleaved to yield multiple copies of the desired RNA oligonucleotide.

57. The method of claim 56 wherein the cleavage is autolytic.

58. The method of claim 56 wherein the desired RNA oligonucleotide is linear.

59. The method of claim 56 wherein the desired RNA oligonucleotide is circular.

60. The method of claim 52 or 56 wherein the desired RNA oligonucleotide is biologically active.

61. The method of claim 60 wherein the biologically active RNA oligonucleotide comprises a catalytic RNA, an antisense RNA, or a decoy RNA.

62. The method of claim 60 wherein the desired oligonucleotide has endonuclease, exonuclease, polymerase, ligase, phosphorylase, dephosphorylase, or protease activity.

63. The method of claim 60 wherein the desired oligonucleotide comprises a ribozyme.

64. The method of claim 63 wherein the ribozyme is a hairpin, hammerhead-motif, or hepatitis delta catalytic ribozyme.

65. The method of claim 60 wherein the biologically active RNA oligonucleotide is capable of intramolecular ligation.

66. The method of claim 65 wherein the biologically active RNA oligonucleotide comprises a hairpin-type ribozyme.

67. The method of claim 63 wherein the desired RNA oligonucleotide is capable of *trans* cleavage.

68. The method of claim 63 wherein the desired RNA oligonucleotide cleaves a target disease-associated RNA, DNA, or protein.

69. The method of claim 52 or 56 wherein the circular oligonucleotide is administered to the organism using direct injection, inhalation, intranasal administration, ocular administration, site-specific incubation or infusion.

70. The method of claim 69 wherein the circular oligonucleotide is administered via direct injection.

71. The method of claim 70 wherein the organism is a mammal and wherein the circular oligonucleotide is administered to the mammal subcutaneously, intramuscularly, or intravenously.

72. The method of claim 69 wherein a gene encoding an effective RNA polymerase operably linked to a promoter is co-introduced into the cell.

73. The method of claim 72 wherein the gene encoding an effective RNA polymerase is administered to the mammal via direct injection.

74. The method of claim 72 wherein the RNA polymerase is T7 or *E. coli* polymerase.

75. The method of claim 52 or 56 wherein the desired RNA oligonucleotide modifies the structure or the function of a target disease-associated DNA, RNA, or protein.

76. The method of claim 75 wherein the desired RNA oligonucleotide comprises a biologically active RNA that cleaves a target disease-associated RNA in *trans*.

77. A method for treating disease in a living organism comprising administering to the organism an RNA oligonucleotide multimer synthesized according to the method of claim 1, wherein the RNA oligonucleotide multimer modifies the structure or function of a target disease-associated molecule.
78. A method for treating disease in a living organism comprising administering to the organism an RNA oligonucleotide synthesized according to the method of claim 16, wherein the RNA oligonucleotide modifies the structure or function of a target disease-associated molecule.
79. The method of claims 77 or 78 wherein the target disease-associated molecule comprises a nucleic acid.
80. The method of claim 79 wherein the target disease-associated nucleic acid is RNA.
81. The method of claim 80 wherein the desired oligonucleotide comprises a biologically active RNA that cleaves a target disease-associated RNA in *trans*.
82. An RNA molecular weight standard kit comprising packaging containing, separately packaged, a population of about 4-50 repeating unit RNA molecules each having a different number of nucleotides such that each successively larger repeating unit RNA molecule differs from the immediately preceding repeating unit RNA molecule by a number of nucleotides equal to the number of nucleotides in a single-stranded circular oligonucleotide template; wherein the circular oligonucleotide template comprises at least one copy of a nucleotide sequence that encodes a ribozyme and a cleavage site for the ribozyme; and wherein the population of repeating unit RNA molecules is synthesized by contacting the circular

oligonucleotide template with an effective RNA polymerase and an effective amount of at least two ribonucleotide triphosphates to yield an RNA oligonucleotide multimer, for a period of time effective to allow partial autolytic cleavage of the RNA oligonucleotide multimer to yield the desired population of repeating unit RNA molecules.

83. The RNA molecular weight standard kit of claim 82 wherein the circular oligonucleotide template has about 15 to 1500 nucleotides.
84. The RNA molecular weight standard kit of claim 83 wherein the circular oligonucleotide template has about 20 to 500 nucleotides.
85. The RNA molecular weight standard kit of claim 82 wherein the RNA molecules range in length from about 50 to  $10^4$  nucleotides.
86. The RNA molecular weight standard kit of claim 85 wherein the RNA molecules range in length from about 50 to 500 nucleotides.
87. The RNA molecular weight standard kit of claim 82 wherein the population of RNA molecules comprises a lyophilized powder.
88. The RNA molecular weight standard kit of claim 82 wherein the population of RNA molecules comprises a buffered solution containing a quenching agent.
89. The RNA molecular weight standard kit of claim 88 wherein the quenching agent is formamide, urea, or EDTA.
90. The RNA molecular weight standard kit of claim 82 wherein the RNA molecules are detectably labeled.

91. A kit for synthesizing RNA molecular weight standards comprising packaging containing, separately packaged, instructions for use and a single stranded circular oligonucleotide template comprising at least one copy of a nucleotide sequence that encodes a ribozyme and a cleavage site for the ribozyme.

92. The kit of claim 91 wherein the circular oligonucleotide template has about 15-1500 nucleotides.

93. The kit of claim 92 wherein the circular oligonucleotide template has about 20-500 nucleotides.

94. The kit of claim 91 wherein the circular oligonucleotide template is devoid of an RNA polymerase promoter.

95. A method for synthesizing an RNA oligonucleotide inside a cell comprising introducing into a cell a single-stranded circular oligonucleotide template comprising at least one copy of a nucleotide sequence complementary to the sequence of the RNA oligonucleotide, such that the circular oligonucleotide is processed intracellularly to yield an RNA oligonucleotide multimer comprising multiple copies of the RNA oligonucleotide.

96. The method of claim 95 wherein the circular oligonucleotide has about 15-1500 nucleotides.

97. The method of claim 95 wherein the cell is a plant cell or an animal cell.

98. The method of claim 95 wherein the cell is a bacterial cell.

99. The method of claim 95 wherein the cell is a mammalian cell.

100. The method of claim 95 further comprising cleaving the RNA oligonucleotide multimer to yield multiple copies of the RNA oligonucleotide.
101. The method of claim 100 wherein the cleavage is autolytic.
102. The method of claim 100 wherein the RNA oligonucleotide is linear.
103. The method of claim 100 wherein the RNA oligonucleotide is circular.
104. The method of claim 100 wherein the RNA oligonucleotide is biologically active.
105. The method of claim 104 wherein the biologically active RNA oligonucleotide comprises a catalytic RNA, an antisense RNA, or a decoy RNA.
106. The method of claim 104 wherein the biologically active RNA oligonucleotide has endonuclease, exonuclease, polymerase, ligase, phosphorylase, dephosphorylase, or protease activity.
107. The method of claim 104 wherein the biologically active RNA oligonucleotide is capable of intramolecular ligation.
108. The method of claim 104 wherein the biologically active oligonucleotide comprises a ribozyme.
109. The method of claim 108 wherein the ribozyme is a hairpin, hammerhead-motif, or hepatitis delta catalytic ribozyme.

110. The method of claim 108 wherein the ribozyme is capable of *trans* cleavage.

111. The method of claim 108 wherein the ribozyme cleaves a target disease-associated RNA, DNA, or protein.

112. The method of claim 104 wherein the biologically active RNA oligonucleotide modifies the structure or the function of a target disease-associated DNA, RNA, or protein.

113. The method of claim 95 wherein a gene encoding an effective RNA polymerase operably linked to a promoter is co-introduced into the cell.

114. The method of claim 113 wherein the RNA polymerase is T7 or *E. coli* polymerase.

115. The method of claim 95 wherein the circular oligonucleotide template is introduced into the cell using direct injection, electroporation, heat shock, calcium phosphate treatment, lipid-mediated delivery, or cation-mediated delivery.

116. The method of claim 95 further comprising implanting the cell into a plant or animal after introducing the single-stranded circular oligonucleotide template into the cell.

117. The method of claim 95 performed in a cell explanted from a plant or animal.

118. The method of claim 117 further comprising implanting the cell into a plant or animal after introducing the single-stranded circular oligonucleotide template into the cell.

119. The method of claim 118 wherein the cell is reimplanted into the plant or animal from which it was explanted.

120. The method of claim 117 wherein the animal is a mammal.

121. The method of claim 95 performed in cell culture.

122. The method of claim 95 performed *in situ* in a living organism.

123. The method of claim 122 wherein the circular oligonucleotide is administered to the organism using direct injection, inhalation, intranasal administration, ocular administration, site-specific incubation or infusion.